Long-Term Adjuvant Therapy of High-Risk Malignant Melanoma with Interferon α2b

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Fifty-three high-risk melanoma patients in stage I and 15 patients in stage II were treated after standard surgical intervention with adjuvant therapy with recombinant interferon α-2b (rIFNα2b) therapy for a total period of 20 months. Concomitant patients (stage I, n = 82; stage II, n = 33) with identical stages and prognostic factors without adjuvant therapy were used to evaluate the efficacy of rIFNα2b therapy. No difference in 5-year relapse incidence and overall survival rates could be detected. However, it appears that patients of both stage I and stage II benefit from long-term adjuvant rIFNα2b therapy, because during the treatment period (20 months), the incidence of relapses was lower in comparison to controls. After stopping treatment the incidence of relapse is equal in treated and control groups. According to the results of our study, we suggest using continuous low-dose rIFNα2b therapy for adjuvant treatment of malignant melanoma. *J Invest Dermatol 95:193S–197S,1990*

The incidence of cutaneous malignant melanoma (MM) is constantly rising. This is probably a result of different exogenic factors, especially exposure of fair skinned caucasians to UV-light, pollution, drug consumption, etc. Additionally, there is some evidence for the involvement of genetic factors in the development of this malignant disease [1,2].

During the 10-year period from 1975 to 1985 the life-time risk for the development of malignant melanoma doubled in the white population. At present the incidence of MM is 0.0039% (1 of 25.800 inhabitants) and is still rising [3]. A similar situation is taking place in middle Europe. During the last 2 decades, despite an increase in the incidence, the mortality rate for MM has dropped, probably due to more frequent and earlier diagnosis. Nevertheless, the death rate within 5 years of MM is still next to that of malignant lung diseases [4], with a crude relative mortality rate of 22.5% [3]. According to international statistical analysis the median survival of melanoma patients who have developed distant metastases is about 6 months [4]. Thus, the disease outcome of metastatic melanoma is fatal and is not significantly changed by the use of a variety of therapeutic interventions, such as single agent and/or poly-chemotherapy [5]. Therefore, at the moment no standard effective chemotherapeutic regimen for metastatic malignant melanoma can be recommended, as the overall response rate to various chemotherapeutic regimens is about 25% [6].

In the last decade several approaches for immunotherapy have been used in advanced MM. Methods investigated include tumor vaccines [7] and biologic response modifiers (BRM) of bacterial and synthetic origin [8]. More recently, natural and recombinant cytokines as well as immunotoxins have been investigated in phase I and II trials [9,10]. Among these BRM recombinant interferon-α (rIFNα) has been extensively applied for treatment of metastatic MM. Reported response rates were in the range of 5%–30% [11,12]. The known antitumoral effect of IFNα, i.e., antiproliferative- and differentiation-inducing activities together with modulating effects on both oncogen expression and immune functions, probably could have contributed to the clinical responses observed in these studies [11,12]. Based on these experiences with IFNα therapy in advanced stages of MM, this agent appears to be a potential candidate for use in early clinical stages. Results of studies with human cell cultures and in animal models [3], as well as response rates achieved in clinical trials with BRM, have pointed out that minimal residual disease is the most realistic base for an effective treatment with immunomodulating agents such as IFNα [14].

According to international statistical analysis, the overall 5-year survival is 69% for stage I and only 36% for stage II MM [4]. In stage I prognostic factors such as tumor thickness and level of invasion are directly related to the outcome of the disease. Therefore, 95% of patients in the good prognosis group (in situ melanoma) have a life expectancy of more than 5 years. However, 5-year survival rates drop dramatically with increasing tumor thickness and a higher level of invasion (35–38%, level V, tumor thickness more than 4 mm [4,15]).

None of the results of adjuvant therapy studies for high-risk melanomas published have shown significant prolongation of recurrence free interval or survival times [16,17]. Therefore, at present

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Abbreviations:
AB: antibodies
ALM: acral lentiginous melanoma
BCG: bacille Calmette Guerin
BRM: biologic response modifiers
DTIC: 5-(3,3-dimethyl-t-triazeno) imidazole-4-carboxamide
ELISA: enzyme-linked immunosorbent assay
IFN: interferon
IFNα: interferon-α
LMM: lentigo maligna melanoma
MM: malignant melanoma
NK: natural killer cell
NM: nodular melanoma
PB: peripheral blood
rIFN: recombinant interferon
rIFNα: recombinant interferon-α
rIFNα2b: recombinant interferon α-2b
SSM: superficial spreading melanoma

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there is no standardized therapy to be recommended for adjuvant treatment of stage I and II MM.

In the present study we investigated the therapeutic usefulness of rIFNα2b applied for 20 months to patients with operated stage I and stage II MM without evidence of disease after surgery. Results were compared with a concomitant melanoma patient population identical in clinical stage and prognostic factors, receiving no adjuvant therapy.

PATIENTS AND METHODS

Patient Selection Fifty-three patients with stage I and 15 patients with stage II malignant melanoma were included to receive long-term rIFNα2b therapy. Eighty-two patients with stage I and 33 patients with stage II were used as concomitant controls to evaluate the therapeutic efficacy of therapy. Upon entry both patients in active treatment groups and those in the concomitant control group were required to have had radical surgery; i.e., wide excision of primary and elective or curative lymph node dissection. According to histopathologic criteria, patients in stage I were required to have a tumor thickness of ≥ 1.5 and a level of invasion at least III. Stage III patients had to have histologically confirmed melanoma at a nodal level. At time of entry patients had to be without evidence of residual disease at local and distant sites as evaluated clinically and by radiologic and sonographic methods. Patients should have a performance status of 100% on the Karnofsky scale. Further inclusion criteria were a leukocyte count of more than $4 \times 10^9/1$, serum creatinin of < 1.7 mg%, and serum urea less than 9 nmol/1. Total bilirubin, ALAT, and ASAT had to be less than the value, which exceeds the upper laboratory range by 50%. Furthermore, patients in the rIFNα2b arm and also in the concomitant control group were required not to receive any other chemo-, immuno-, radio- or hormonal-treatment for the entire treatment and observation period. Verbal consent was obtained from all patients treated with rIFN.

Study Design and Therapy This is a single agent study in which patients were randomly selected to receive rIFNα2b-therapy. Concomitant MM patients with identical clinical stages and prognostic variables were used as a control group to evaluate the therapeutic efficacy of this therapy. The latter group did not receive any therapy except surgery and was controlled with identical methods in the respective time intervals. Study end points were relapse incidence and estimation of relapse free interval and survival. Recombinant rIFNα2b (Introna, Schering) was reconstituted with sterile water and injected subcutaneously close to the primary surgery area on an outpatient basis. Patients received initially for 3 months 3×10^6 rIFNα2b 3 times weekly; from month 4 to 9, 3×10^6 IU rIFNα2b 2 times weekly; and from month 10 to 20, 2×10^6 IU rIFNα2b 2 times weekly. Patients were trained to inject the drug by themselves. Injections were usually in the evening.

Evaluation of Toxicity Subjective and objective side effects were evaluated initially on a weekly base and later at 3-month intervals base and graded according to the World Health Organization toxicity scale [18]. In case of severe toxicity (grade 3 and 4), rIFNα2b dose could be reduced by 50% or treatment interrupted until normalization of all pathologic events.

Pretreatment Evaluation and Follow-Up Before initiation of therapy all patients underwent a complete medical history and physical examination. Laboratory studies included complete blood count with differential and platelet count, HTK, Hbg, BUN, creatinine, and liver function tests. These tests were repeated in monthly intervals during the initial therapy phase and later in 3-month intervals. Pretreatment examinations also included chest x ray and abdominal and lymph-node sonography and were repeated at the respective time intervals during therapy. Bone, spleen, liver-scan, and CT-investigations were performed on requirement. Furthermore, natural killer cell (NK) activity of peripheral blood mononuclear cells was determined in monthly intervals during the 20-month treatment period.

Table I. Stage I Patients' Characteristics and Histopathologic Criteria

	IFN-Group (median age, 48; range, 21–70)		Control Group (median age, 52; range, 19–67)		
Criteria	n	%	n	%	n.s.[a]
Sex					
female	20	37.8	41	50.0	
male	33	62.2	41	50.0	n.s
total	53	100.0	82	100.0	
Type of tumor					
SSM	14	26.4	20	24.4	
NM	32	60.3	48	58.6	
LMM	0	0.0	1	1.2	n.s.
ALM	4	7.5	2	2.4	
unclassified	3	5.8	11	13.4	
total	53	100.0	82	100.0	
Clark's level					
III	10	18.9	25	30.5	
IV	40	75.5	50	60.9	n.s.
V	3	5.6	7	8.5	
total	53	100.0	82	100.0	
Tumor thickness					
1.50–2.25	21	39.6	29	35.4	
2.26–3.00	18	34.0	19	23.2	n.s.
>3.01	14	26.4	34	41.4	
	53	100.0	82	100.0	

[a] ns, not significant.

NK-Assay This assay was performed as previously described [19]. PB mononuclear cell fraction was obtained by Ficoll-Hypaque centrifugation and investigated in a 4-h ^{51}Cr-release assay using K562 target cells. ^{51}Cr-release of triplicate samples at different E:T ratios (40:1, 20:1, 10:1) was measured in a gamma-counter and expressed by the formula $\%cytoxicity = (cpm_{test} - cpm_{spontan}) / (cpm_{may} - cpm_{spontan})$ [20].

Neutralizing Antibodies Representative serum samples were controlled for the development of anti-rIFNα2b antibodies (AB) by means of a standard antiviral neutralization bioassay and occasionally by a competition ELISA (dye uptake test competition ELISA) [21].

Statistical Analysis Survival curves were calculated according to the method of Kaplan and Mayer expressing the time from diagnosis of stage I and II disease to the date of first relapse or to the last date of contact or death [22]. Treatment groups were compared to the control group regarding disease-free survival and overall survival using the generalized Wilcoxon method. p values < 0.05 were considered to be of significance [23,24].

RESULTS

Patient Characteristics Altogether 53 patients with stage I and 15 patients with stage II have been entered on the rIFNα2b arm. The control group used for data analysis and evaluation of efficacy of therapy consisted of concurrently diagnosed and surgically treated melanoma patients; 83 patients were in stage I and 33 patients in stage II. There was no significant difference (chi-square analysis and t test) according to sex, age, and histopathologic criteria, including levels of invasion and tumor thickness (Table I). All patients had received standard surgical procedures as used for treatment of stage I and II malignant melanoma, i.e., wide local excision of primary elective (stageI) or curative (stageII) lymph-node dissection.

Treatment Results The comparison of Kaplan-Mayer curves of relapse free intervals within a 5-year observation period between

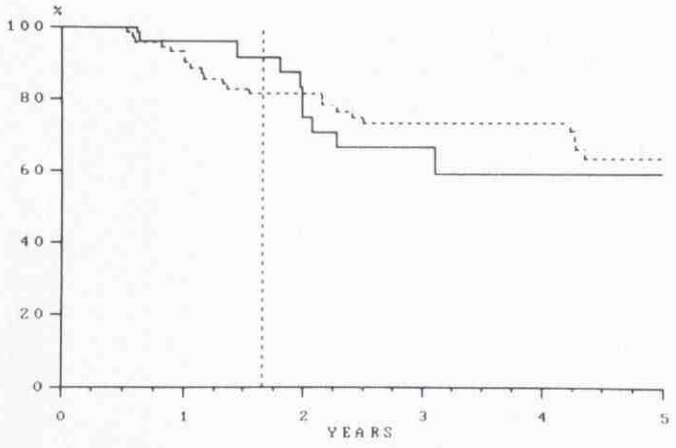

Figure 1. Lifetable analysis of relapse-free intervals (stage I MM patients). *Solid line*, rIFNα2b-treated patients; *dashed line*, untreated control patients. *Dashed reference line*, end of therapy after 20 months.

patients in stage I receiving rIFNα2b therapy and those without adjuvant therapy did not show any statistically significant difference according to the generalized Wilcoxon method. Overall relapse incidence within a 5-year period in stage I was 10 out of 53 rIFNα2b treated patients and 22 out of 82 concomitantly controlled patients. However, survival curves demonstrate that in the first year of rIFNα2b therapy only 2 out of 53 stage I patients relapse and until the end of therapy one additional patient developed metastases. The probability of relapse-free survival after 20 months for this group was 92%. In the control group 12 patients developed metastases within the first 20 months and the probability of relapse-free survival after this time period was 81.4% [Fig 1]. In stage II patients, again, there was no significant difference between the treated group and the control group regarding relapse-free survival. After an observation period up to 5 years, six patients in the rIFNα2b-treated group (n = 15) and 15 patients out of the control group (n = 33) relapsed. However, within the treatment period of 20 months three patients in the rIFNα2b-arm and eight patients in the control arm relapsed. The probability of relapse-free survival after 20 months was 79.4% and 73.3%, respectively for these groups [Fig 2].

The pattern of metastases in relapsing patients is in accordance with the known localization of organ metastases in melanoma disease [3]. Out of 53 stage I rIFNα2b treated patients, four developed lung metastases, two multiple subcutaneous metastases, two distant lymph node metastases, one multiple liver metastases, and one multiple bone metastases. Two patients out of this group died (23 months and 43 months after initiation of treatment). The metastases pattern in the stage I concomitant control group was similar to that of the rIFNα2b group, except for brain metastases, as four patients developed multiple brain lesions. Eleven patients in this group died during the observation period, with a median survival time of 28 months (12–60 months).

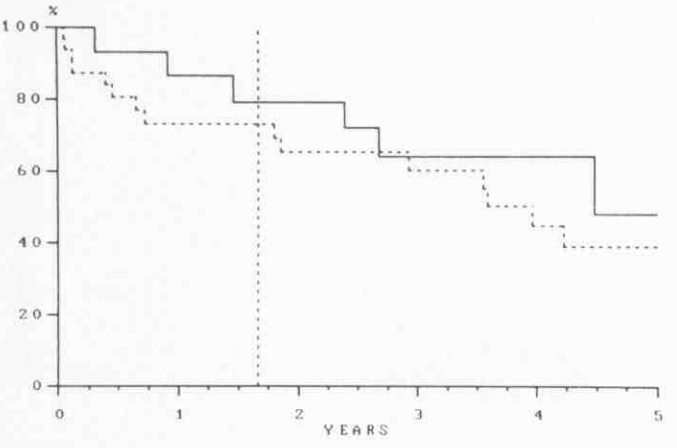

Figure 2. Lifetable analysis of relapse-free intervals (stage II MM patients). *Solid line*, rIFNα2b treated patients; *dashed line*, untreated control patients. *Dashed reference line*, end of therapy after 20 months.

Metastases in rIFNα2b-treated stage II patients were documented in the following localizations: multiple subcutaneous tumors (n = 2), distant lymph node metastases (n = 2), brain metastases (n = 1), and lung metastases (n = 1). Four patients died within 5 years, with a median survival time of 28.5 months. In the concomitant control patients' group the metastatic pattern was as follows: multiple subcutaneous (n = 2), lymph node metastases (n = 3), multiple brain metastases (n = 2), lung metastases (n = 5), and intestinal metastases (n = 2). Six patients died within the observation period, and their median survival time was 18 months. Overall survival time was analyzed using the method of Kaplan and Mayer, and differences between treated and control groups were estimated by the generalized method of Wilcoxon as described above. A significant difference between the rIFNα2b-treated group and the control group could not be detected (data not shown) in stage I or II patients.

Side Effects In almost all patients rIFNα2b therapy was well tolerated, resulting only in WHO grade I and II toxicity. Initially, 98% of the patients developed flu-like symptoms, 51% nausea and vomiting, and the rest intestinal symptoms (18%). Flu-like symptoms could be effectively controlled by acetaminophen or paracetamol. After at least 2 months of rIFNα2b therapy patients developed tachyphylaxis to these symptoms. Hematologic and other laboratory parameters were only transient and to some extent influenced by rIFNα therapy. Leukopenia, grade I and II, was observed in 15% of patients and thrombopenia was observed in 3% of patients receiving rIFNα2b therapy. Some influence on hemoglobin, serum transaminases, and alkaline phosphatase was detected in a small percentage of patients. Anorexia (20%) and hair loss grade I (7%) were the only observed toxicities that developed during long-term rIFNα2b therapy. Incidence and severity of side effects did not require dose reduction or discontinuation of rIFNα2b therapy in any of the patients. There has been no evidence for the development of IFNα-neutralizing antibodies in any of the patients investigated by these methods.

Immunmonitoring Serial determination of NK activity of patients receiving rIFNα2b therapy and concomitant control patients revealed continuous higher but not significantly different levels in treated patients in comparison to controls [Fig 3].

DISCUSSION

There exists no standardized treatment regimen to be used as adjuvant therapy in patients with high-risk stage I MM and in patients with lymph node metastases of MM. So far all attempts to improve relapse rates and overall survival have failed. Neither the use of chemotherapeutic agents such as DTIC or polychemotherapy nor BRM [especially of bacterial origin such as Bacille Calmette Guerin (BCG), Corynebacterium parvum, or combinations of both or tumorvaccines] have shown any significant advantage in comparison to patients who received no adjuvant therapy following standardized surgical procedures [8,16,17]. Based on the known therapeutic efficacy of recombinant rIFNα in patients with advanced MM [11,12,14] and on our own experiences [28], we have investigated the usefulness of rIFNα2b applied for 20 months as adjuvant therapy to patients with high-risk MM in stages I and II. Kaplan Mayer analysis suggests a benefit regarding relapse-free interval in patients with stage I malignant melanoma during the rIFNα2b treatment period (up to 20 months) in comparison to patients receiving no adjuvant therapy. However, according to statistical analysis of the probability for relapse incidence within a 5-year period, there was no significant difference between these two groups. These results suggest that rIFNα2b therapy has altered the natural history of

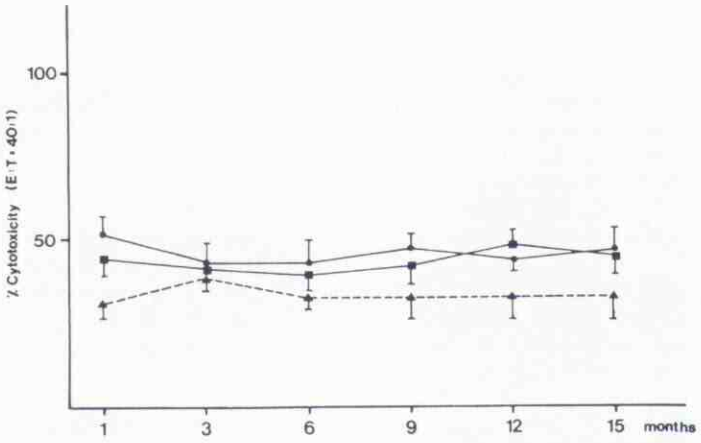

Figure 3. Peripheral blood NK-cell activity of rIFNα2b-treated stage I *(closed circle)*, stage II *(closed square)*, and untreated control patients *(closed triangle)*.

malignant melanoma in stage I for a least a 20-month period. In stage II patients a similar tendency has been observed, suggesting again that adjuvant rIFNα2b therapy might be of benefit to this patient group.

One can only speculate on possible mechanisms underlying this suggested therapeutic effect of rIFN therapy at present. Ample evidence that rIFNα has a significant antiproliferative effect on human melanoma cells in vitro and probably also in vivo exists. The latter conclusion can be drawn from clinical studies where rIFN therapy has resulted in significant tumor remissions in patients with metastatic MM [11,12,14]. Our own study in metastatic melanoma, using a dose escalation schedule, has also documented some therapeutical efficacy of rIFNα [24]. Additionally, it might be postulated that rIFNα acts as an immunmodulating agent in this therapy setting [24,25]. The results of NK assays performed in patients receiving rIFNα2b therapy are suggestive for such an effect, showing some stimulation of this activity during the course of therapy, i.e., continuous higher levels were observed in rIFNα2b treated patients in comparison to controls.

Additionally, it should be emphasized that we never observed significant suppression of this immune function during low-dose rIFNα therapy, as has been recently reported by others [26]. These differences in the effects of rIFN therapy on immunfunction might be a result of different patient populations studied. The role of adjuvant rIFN-gamma therapy in MM has been recently investigated in a pilot study which included 25 patients with stage I high-risk MM and 15 patients with lymph node metastases. The authors found a tumor progression in 36% and 32%, respectively, of patients within the first year, demonstrating no advance of adjuvant IFN-gamma therapy [27]. Our results, however, demonstrate that in the first year of rIFNα-therapy only two out of 53 stage I patients developed metastases. At the end of a 20-month rIFNα2b therapy period the probability of relapse-free survival is 92%. In the untreated patients population, 12 relapsed within the first 20 months, resulting in a relative relapse-free survival rate of 81.4%.

These data suggest that patients with high-risk MM might benefit from long-term rIFNα2b therapy, as discontinuation of rIFNα2b application has resulted in a more frequent incidence of relapses. Thus, one might conclude that continuous exposure to exogenous rIFNα may be important in this malignant disease to maintain therapeutic efficacy. The experience with melanoma cell cultures (continuous exposure is required for optimal antiproliferative effects of this agent) might also contribute to this notion [28]. Additionally, the proposed immunomodulating activities of IFNα in vivo, which include the modulation of surface antigen expression (tumor-associated antigens, histocompatibility antigens) together with the stimulation of killer cell activities (T-lymphocytes, NK-cells, and macrophages) might also be dependent on continuous application of exogenous IFNα [29]. There is evidence that melanoma patients have a stage-dependent deficiency in NK activity and in the production of endogenous IFNα [30]. Thus, long-term rIFNα therapy, as used in our study, might contribute to a normalization of an impairment of immunfunction and endogenous IFN production.

Recently, a WHO-sponsored multicenter randomized trial for treatment of stage II malignant melanoma patients with IFNα was initiated.* This study is in accordance with our findings and future plans to use IFNα as long as the patient remains relapse free will be attempted. Based on the experience of our present study, which suggests some therapeutic activity of long-term adjuvant rIFNα2b therapy and demonstrates the absence of significant toxicity, we have initiated a prospective randomized trial for treatment of melanoma patients with high risk for relapse, comparing rIFNα2b therapy with no further therapy.

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